

In the Claims:

1. (Currently Amended) A method for ~~large-scale~~, continuous production of ~~large quantities of an individual Class I MHC complexes molecule~~, comprising the steps of:

isolating total RNA from a source and reverse transcribing mRNA present in the total RNA to form cDNA, wherein the total RNA contains mRNA for at least one MHC Class I heavy chain allele and reverse transcribing the mRNA ~~forms~~ to form a cDNA encoding a desired MHC Class I heavy chain molecule allele;

creating a truncated PCR product encoding the desired MHC Class I heavy chain molecule allele by PCR amplification of the cDNA encoding the desired MHC Class I ~~allele~~ heavy chain molecule wherein the PCR product does not encode the transmembrane and cytoplasmic domains of the desired ~~Class I MHC~~ heavy chain molecules, thereby producing a PCR product that encodes an individual, soluble Class I MHC heavy chain molecule;

cloning the PCR product into a mammalian expression vector to create a construct;

electroporating or transfecting the construct into a suitable host cell; and inoculating a hollow fiber bioreactor unit with the host cell containing the construct for ~~large-scale~~ continuous production of the soluble individual Class I MHC complexes having the desired MHC Class I

heavy chain molecule associated with native beta-2-microglobulin  
and further wherein the soluble individual Class I MHC complexes  
are loaded with endogenously produced peptides. ~~molecule such~~  
~~that large quantities of the soluble individual Class I MHC molecule~~  
~~are produced.~~

2. (Currently Amended) The method of claim 1 wherein fresh media, oxygen and glucose are fed into ~~said~~ the low fiber bioreactor unit at a rate to maintain optimum cell growth and to maintain harvest rates at a desired level of soluble individual Class I MHC ~~molecules~~ complexes.

3-4. (Canceled)

5. (Currently Amended) The method of claim 1 further comprising the step of harvesting the soluble individual Class I MHC ~~molecules~~ complexes from the hollow fiber bioreactor unit ~~by a continuous harvest method.~~

6. (Currently Amended) The method of claim 1 wherein, ~~in the step of electroporating or transfecting the construct into a host cell,~~ the host cell is a human host cell that lacks expression of Class I MHC ~~molecules~~ complexes.

7. (Currently Amended) The method of claim 1 wherein, ~~in the step of cloning the PCR product into a mammalian expression vector,~~ the mammalian expression vector contains a promoter that facilitates expression of the PCR product.

8. (Currently Amended) The method of claim 1 wherein, ~~in the step of isolating total RNA from a source,~~ the source of the total RNA is selected from the group consisting of a virus transformed cell line and an immortalized cell line.

9. (Currently Amended) The method of claim 1 wherein, ~~in the step of creating a truncated PCR product encoding the desired MHC Class I allele,~~ one of the primers used to create the truncated PCR product is designed to add a tail to the individual Class I MHC desired MHC Class I heavy chain molecule expressed from the PCR product.

10. (Currently Amended) The method of claim 9 further comprising the steps of harvesting the soluble individual Class I MHC ~~molecules~~ complexes from the hollow fiber bioreactor unit ~~by a continuous harvest method~~ and purifying the soluble individual Class I MHC ~~molecules~~ complexes using the tail attached to the soluble individual Class I MHC heavy chain molecules.

11. (Currently Amended) A method for ~~large-scale~~, continuous production of ~~large quantities of an~~ individual Class I MHC ~~molecule~~ complexes, comprising the steps of:

isolating total RNA from a source and reverse transcribing mRNA present in the total RNA to form cDNA, wherein the total RNA contains mRNA for at least one MHC Class I heavy chain allele and reverse transcribing the mRNA ~~forms~~ to form a cDNA encoding a desired MHC Class I ~~allele~~ heavy chain molecule;

creating a truncated PCR product encoding the desired MHC Class I ~~allele~~ heavy chain molecule by PCR amplification of the cDNA encoding the desired MHC Class I ~~allele~~ heavy chain molecule wherein the PCR product does not encode the transmembrane and cytoplasmic domains of the desired MHC Class I ~~molecules~~ heavy chain molecule, thereby producing a PCR product that encodes an individual, soluble Class I MHC heavy chain molecule;

cloning the PCR product into a mammalian expression vector to create a construct;

electroporating or transfecting the construct into a suitable host cell; and inoculating a hollow fiber bioreactor unit with the host cell containing the construct for ~~large-scale~~ continuous production of the soluble individual Class I MHC ~~molecule~~ complexes having the desired MHC Class I heavy chain molecule associated with native beta-2-

microglobulin and further wherein the soluble individual Class I MHC complexes are loaded with endogenously produced peptides, wherein fresh media, oxygen and glucose are fed into said the hollow fiber bioreactor unit at a rate to maintain optimum cell growth and to maintain harvest rates at a desired level of soluble individual Class I MHC ~~molecules such that large quantities of the soluble individual Class I MHC molecule are produced~~ complexes; and

harvesting the soluble individual Class I MHC ~~molecules~~ complexes from the hollow fiber bioreactor unit ~~by a continuous harvest method.~~

12. (Currently Amended) A method for ~~large scale,~~ continuous production of ~~large quantities of an individual Class I MHC molecule~~ complexes, comprising the steps of:

isolating total RNA from a source and reverse transcribing mRNA present in the total RNA to form cDNA, wherein the total RNA contains mRNA for at least one MHC Class I heavy chain allele and reverse transcribing the mRNA ~~forms to form~~ to form a cDNA encoding a desired MHC Class I ~~allele~~ heavy chain molecule;

creating a truncated PCR product encoding the desired MHC Class I ~~allele~~ heavy chain molecule by PCR amplification wherein the PCR product does not encode the transmembrane and cytoplasmic domains of

the desired MHC Class I ~~molecules~~ heavy chain molecule, thereby producing a PCR product that encodes an individual, soluble Class I MHC heavy chain molecule, wherein one of the primers utilized in the PCR amplification is designed to add a tail to the individual, soluble Class I MHC heavy chain molecule expressed from the PCR product;

cloning the PCR product into a mammalian expression vector to create a construct;

electroporating or transfecting the construct into a suitable host cell;

inoculating a hollow fiber bioreactor unit with the host cell containing the construct for ~~large-scale~~ continuous production of the soluble individual Class I MHC ~~molecule~~ complexes having the desired MHC Class I heavy chain molecule associated with native beta-2-microglobulin and further wherein the soluble individual Class I MHC complexes are loaded with endogenously produced peptides, wherein fresh media, oxygen and glucose are fed into ~~said the~~ the hollow fiber bioreactor unit at a rate to maintain optimum cell growth and to maintain harvest rates at a desired level of soluble individual Class I MHC ~~molecules such that large quantities of the soluble individual Class I MHC molecule are produced~~ complexes;

and

harvesting the soluble individual Class I MHC ~~molecules~~ complexes from

the hollow fiber bioreactor unit ~~by a continuous harvest method~~.

13. (Currently Amended) The method of claim 12 further comprising the step of purifying the soluble individual Class I MHC ~~molecules~~ complexes using the tail attached to the soluble individual Class I MHC heavy chain molecules .

14. (Currently Amended) A method for production of an individual Class I MHC ~~molecule~~ complexes, comprising the steps of:

isolating total RNA from a source and reverse transcribing mRNA present in the total RNA to form cDNA, wherein the total RNA contains mRNA for at least one MHC Class I heavy chain allele and reverse transcribing the mRNA ~~forms~~ to form a cDNA encoding a desired MHC Class I allele heavy chain molecule;

creating a truncated PCR product encoding the desired MHC Class I allele heavy chain molecule by PCR amplification of the cDNA encoding the desired MHC Class I allele heavy chain molecule wherein the PCR product does not encode the transmembrane and cytoplasmic domains of the desired class I MHC ~~molecules~~ heavy chain molecule, thereby producing a PCR product that encodes an individual, soluble Class I MHC heavy chain molecule;

cloning the PCR product into a mammalian expression vector to create a construct;

electroporating or transfecting the construct into a suitable host cell; and inoculating a hollow fiber bioreactor unit with the host cell containing the construct for production of the soluble individual Class I MHC ~~molecule~~ complexes having the desired MHC Class I heavy chain molecule associated with native beta-2-microglobulin and wherein the soluble individual Class I MHC complexes are loaded with endogenously produced peptides.